Amendments to the Specification:

Please replace the paragraph beginning at page 5, line 3, which starts with "Figure 1 lists" with:

Figure 1A (1A-1 to 1A-60) lists the atomic structure coordinates for unphosphorylated JNK3 in complex with MgAMP-PNP as derived by X-ray diffraction from a crystal of that complex. The following abbreviations are used in Figure 1:

"Atom type" refers to the element whose coordinates are measured. The first letter in the column defines the element.

"X, Y, Z" crystallographically define the atomic position of the element measured.

"B" is a thermal factor that measures movement of the atom around its atomic center.

"Occ" is an occupancy factor that refers to the fraction of the molecules in which each atom occupies the position specified by the coordinates. A value of "1" indicates that each atom has the same conformation, i.e., the same position, in all molecules of the crystal.

Please replace the paragraph beginning on page 5, line 20, which starts with "Fig 1a" with:

Fig 1[[a]]B is a structure-based sequence alignment of JNK3, ERK2, p38 and cAPK (SEQ ID NOs: 1, 7, 8, and 9, respectively).

Please replace the paragraph beginning on page 30, line 30, which starts with "A BLAST search" and continues through page 31 with:

B2

A BLAST search of the EST database using the published JNK3α1 cDNA [S. Gupta et al. (1996)] as a query identified an EST clone (#632588) that contained the entire coding sequence for human JNK3α1. Polymerase chain reactions (PCR) using *pfu* polymerase (Strategene) were used to introduce restriction sites into the cDNA for cloning into the pET-15B expression vector at the NcoI and BamHI sites for expression of the protein in *E. coli*. Due to the poor solubility of the expressed full length protein (Met 1-Gln 422), an N-terminally truncated protein starting at Ser residue at position 40 (Ser 40), corresponding to Ser 2 of JNK1 and JNK2 proteins [S. Gupta et al. (1996)], preceded by Met (initiation) and Gly residues, was produced. The Gly residue was added in order to introduce an NcoI site for cloning into the expression vector. Further, systematic C-terminal truncations were performed by PCR to identify a construct that give rise to diffraction-quality crystals. This construct, which was prepared by PCR using deoxyoligonucleotides 5' GCTCTAGAGCTCCATGGGCAGCAAAAGCAAAGTTGACAA 3' (SEQ ID NO: 2; forward primer with initiation codon underlined) and 5' TAGCGGATCCTCATTCTGAA TTCATTACTTCCTTGTA 3' (SEQ ID NO: 3; reverse primer with stop codon underlined) as primers and confirmed by DNA sequencing, encodes amino acid residues Ser40-Glu402 of JNK3α1, preceded by Met and Gly residues, was used for structural studies described in this paper. Control experiments indicated that the truncated JNK3 protein has an equivalent kinase activity towards myelin basic protein when activated with an upstream kinase MKK7 in vitro (unpublished results).

Please replace the paragraph beginning on page 35, line 8, which starts with "The crystal structure" with:

B3

The crystal structure includes unphosphorylated JNK3 (residues 45-211, 217-373, 379-400), adenylyl imidodiphosphate (AMP-PNP, an ATP analogue) and two Mg^{2+} ions. Electron density for residues 40-44, 212-216 and 401-402 is not seen, and these amino acids are presumed disordered. The MAP kinase homologous region of JNK3 (Phe48-Glu397) is 45% identical in amino acid sequence to Erk2 and 51% to p38, whose structures have been reported (F. Zhang et al. (1994); K. P. Wilson et al. (1996); Fig 1). As expected, the overall architecture of JNK3 is highly similar to that of Erk2 and p38. The N-terminal lobe (residues 45-149, and 379-400) of JNK3 contains mostly beta-strands, whereas the C-terminal lobe (residues 150-211, 217-374) is predominantly alpha-helical. A deep cleft between the two domains comprises the ATP binding site, where the glycine-rich sequence of the enzyme (GSGAQGIV) (SEQ ID NO: 5) forms a well defined β strand-turn-β strand structure over the nucleotide. The MAP kinase insertion in the C-terminal domain is 12 residues longer in JNK3 than in Erk2 and p38, resulting in the N-terminal extension of helix αH and an extra 3_{10} helix, denoted 3/10(2)L14 between αH and α3L14 (Fig. 2a). We refer to this 12-residue insertion as "the JNK insertion" since it is present in all c-Jun N-terminal kinases [S. Gupta et al., (1996)].

Please replace the paragraph beginning on page 40, line 3, which starts with "The catalytic core" and continues through page 41 with:

B4

The catalytic core of protein kinases contains a nucleotide binding sequence Gly-X-Gly-X-X-Gly-X-X (SEQ ID NO: 3) that is referred to as "the glycine-rich phosphate anchor loop" due to its structural feature and role in the nucleotide binding [D. R. Knighton et al., Science, 253, pp. 407-13 (1991)]. The glycine-rich loop is well defined in JNK3, and superimposes well with that of cAPK, with an rms deviation 0.54 Å for the protein main chain atoms from Ile70 to Ser79 (Fig.4). The glycine-rich sequence Gly71-Ser-Gly73-Ala-Gln-Gly76-Ile-Val78 (SEQ ID NO: 4) forms a flap over the nucleotide, covering it almost completely. The adenine base of the nucleotide is deep in the back of the domain interface, with its amino group (N6) making a hydrogen bond to the backbone carbonyl of Glu147, and N1 to the backbone amide of Met149. Non-polar interactions are also found at both sides of the purine ring, including Ile70 and Val78 from the glycine-rich flap on one side and Val196 from 7 on the other. The ribose O2' and O3' hydroxyls form a hydrogen-bonding network to the side chain of Asn152 and the carbonyl group of Ser193. The triphosphate group is tightly connected via hydrogen bondings, involving directly or indirectly, most of the invariant amino acids of protein kinases. Hydrogen bonds to phosphate oxygen atoms are formed by main chain amides of Gln75 and side chains of Gln75 and Lys93. Two magnesium ions (M1 and M2) are observed in the JNK3-MgAMP-PNP complex. The side

chain carbonyl group of Asn194 is in close contact with M1 metal ion, which in turn bridges the oxygens of the α and γ phosphoryl groups of AMP-PNP. Asp207 interacts through water molecules with M2, which is bound to the β and γ phosphoryl group oxygens, while in cAPK, the corresponding residue (Asp184) directly coordinates both M1 and M2. This significant difference appears to be due to the inactive conformation of the JNK3 enzyme. An important role in metal chelation has been proposed for Asp184 in cAPK which requires direct interaction of the aspartic residue with the metal ion [D. R. Knighton et al., Science, 253, pp. 407-13 (1991); D. Bossemeyer et al. (1993)]. Asp207 is located at a loop called the "DFG loop" preceding the disordered β9 in unphosphorylated JNK3. The structure of JNK3 suggests that upon phosphorylation, the refolding the phosphorylation lip and domain rotation should bring Asp207 closer to the nucleotide to allow its direct interaction with the metal ion.

B4 cont'd

Please replace the paragraph beginning on page 43, line 16, which starts with "Figures 1a-5" with:

B5

Figures 1[[a]]B-5 further depict the structure of the JNK3/MgAMP-PNP complex. Thus, Fig 1[[a]]B depicts the structure-based sequence alignment of JNK3 [S. Gupta et al., (1996)], ERK2 [T. G. Boulton et al., Cell, 65, pp. 663-75 (1991)], p38 [J. C. Lee et al., Nature, 372, pp. 739-46 (1994)] and cAPK [M. D. Uhler, Proc. Natl. Acad. Sci. USA, 83, pp. 1300-04 (1986)]. The amino acid sequences of human JNK3, human ERK2, human p38 kinase, and murine cAPK are aligned based on structural

similarity. The divergent N- and C-terminal regions of Erk2, p38 and cAPK are not shown. N- and C-terminal residues that are not included in the truncated JNK3 (JNK3: residues Ser40-Glu402) for crystallographic studies are denoted by lowercase letters. Residues in italic are not included in the model. Subdomains are labeled by Roman numerals according to S. K. Hanks et al., Science, 241, pp. 42-52 (1988). The secondary structural elements for JNK3 are indicated above the sequences (nomenclature as for Fig 2a), with open boxes designating $\alpha\alpha$ helices and 3/10 helices and open arrows for $\beta\beta$ strands. Disordered regions are indicated with dashed lines. Both JNK3 and cAPK sequence numbering are shown. Phosphorylation sites in the phosphorylation lip are denoted by an asterisk. JNK3 residues that differ from JNK1 and JNK2 are highlighted in bold.

B5 amd

Please replace the paragraph beginning on page 47, line 12, which starts with "To determine IC_{50}" with:

B6

To determine IC_{50} of the compound binding to JNK3, the kinase activity of JNK3 was monitored by coupled enzyme assay. In this assay, for every molecule of ADP generated by the JNK3 kinase activity one molecule of NADH is converted to NAD which can be conveniently monitored as an absorbance decrease at 340 nm. The following are the final concentrations of various reagents used in the assay: 100 mM HEPES buffer, pH 7.6, 10 mM $MgCl_2$, 25 mM β-glycerophosphate, 30 μM ATP, 2 mM phosphoenolpyruvate, 2 μM pyruvate kinase, 2 μM lactate dehydrogenase, 200 μM NADH, 200 μM EGF receptor peptide

KRELVEPLTPSGEAPNQALLR (SEQ ID NO: 6), and 10 nM activated JNK3. First, all of the above reagents with the exception of ATP were mixed and 175 μl aliquots were placed per well of 96-well plate. A 5 μl DMSO solution of the compound was added to each well, mixed, and allowed to stand at 30°C for 10 minutes. Typically about 10 different concentrations of the compound were tested. The reactions were initiated with the addition of 20 μl of ATP solution. Absorbance change at 340 nm were monitored as a function of time. IC_{50} is obtained by fitting the rates vs. compound concentration data to a simple competitive inhibition model.